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PHARMACOLOGY

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8885

JUL 23 1973

Date: July 16, 73

Application for Research Grant
(Use extra pages as needed)

1. Principal Investigator (give title and degrees): Leonide Goldstein, D. Sc. Associate Professor of Psychiatry, College of Medicine & Dentistry of New Jersey Judith M. Nelsen, Ph. D. Instructor in Psychiatry. College of Medicine & Dentistry of New Jersey.

2. Institution & address: Institute for Mental Health Sciences CMDNJ Rutgers Medical School. P.O. Box 101 Piscataway, NJ 08854

3. Department(s) where research will be done or collaboration provided: Department of Psychiatry

4. Short title of study: Behavioral and Electrophysiological Effects of the "Chronic Nicotine State" in Rats.

5. Proposed starting date: January 1, 1974 (Actually, proposed renewal starting

6. Estimated time to complete: One year

7. Brief description of specific research aims: See attached pages.

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8. Brief statement of working hypothesis:

2.

' Chronic nicotine treatment produces shifts in the balance between reticular formation and limbic influences on arousal, resulting in a state of enhanced "motivational arousal" and reduced "drive arousal". Such changes in brain functional mechanisms should produce the qualitative state of arousal appropriate for engaging in goal-directed behavior. Further, the shift in the balance between the subcortical system influencing arousal (in the "chronic nicotine state") should modify both the behavioral and electrophysiological effects of psycho-active drugs.

9. Details of experimental design and procedures (append extra pages as necessary)

See attached pages.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location). The unit is comprised of 4 specially designed rooms, 2 of which are 10.5' x 16.5' and 2 are 10' x 10'. There are also 3 offices, 10.5' x 12.5'. One of the larger laboratory rooms is equiped for behavioral measurements while the other larger laboratory is equiped for electroencephalographic recordings. All the necessary equipment for this proposal is available largely purchased with funds previously allocated by the Council.

Animal care and maintenance is provided on a per diem basis at the Vivarium of the Medical School, a short distance away. There is an underground tunnel between the 2 buildings. A full time Veterinarian is present.

A part-time secretary is available. We have a Model 1766 Monroe automatic Desk Computer with a card reader and programs for most computations needed for the project.

11. Additional facilities required As mentioned above these are available.

12. Biographical sketches of investigator(s) and other professional personnel (append).

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).
See enclosed list. Reprints of 2 articles are enclosed. More will be sent as soon as they are available.

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R: REDACTED MATERIAL

Second

14. First year budget:

- A. Salaries (give names or state "to be recruited")
 Professional (give % time of investigator(s)
 even if no salary requested)

Leonide Goldstein, D. Sc.

% time _____

Amount _____

Judith M. Nelsen, Ph. D.

10

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Technical

Kathleen Pelley, B.A.

100

REDACTED

Sub-Total for A _____

B. Consumable supplies (by major categories)

Food pellets.....	100.00
Chart paper.....	100.00
Behavioral modules.....	200.00
Chemicals and drugs.....	100.00
Maintenance of computer.....	200.00

Sub-Total for B _____ 700.00

C. Other expenses (itemize)

Travel to 2 Meetings for P.I. 500.00
 Travel to 1 Meeting for co-P.I. 266.00

Sub-Total for C _____ 766.00

Running Total of A + B + C

REDACTED

D. Permanent equipment (itemize):

None

t/s
gh

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements

Sub-Total for D _____

E _____ 4,350.00

Total request

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Salaries	Consumable Suppl	Other Expenses	Permanent Equip	Indirect Costs	Total
Year 2					
Year 3					

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16. Other sources of financial support.

List financial support from all sources, including own institution, for this and related research projects

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Effects of a Prostaglandin analogue on brain electrical activity and behavior	Office of Naval Research. Contract # 14-73-C0203	16,000	8/1/73 - 7/31/74
Rutgers Polydrug Treatment Research Project (Consulting Psycho-Pharmacologist)	PHS	134,438	7/1/73 - 7/1/74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Microwave radiations: Effects on brain and behavior	PHS RL 01047-01	52,923	1/1/74-12/31/74

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Leonide Goldstein, D. Sc.Signature Leonide Goldstein Date 7/16/73Telephone 201 Area Code 932 Number 4416 Extension

Responsible officer of institution

Typed Name Stanley S. Bergen, Jr., M.D.Title PresidentSignature Stanley S. Bergen, Jr., M.D. Date 7/14/73Telephone (201) 877-4400 Area Code " Number " Extension "

Checks payable to
College of Medicine & Dentistry of N.J.
Rutgers Medical School
Mailing address for checks
P.O. Box 101
Piscataway, N.J. 08854

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7. (a) Our previous application to The Council for support (dated October 20, 1972) outlined in some detail the specific aims of the proposed studies. However, it might be useful to review these aims in light of the experimental objectives which have already been achieved. On the basis of studies carried out under past grants from The Council, it was reported that following chronic nicotine treatment there occur certain changes in the features of the electrophysiological mechanisms of arousal. These changes were interpreted as being indicative of a shift from the "classical" arousal mechanism [involving the mesencephalic reticular formation (RF)] to arousal mediated by the limbic system (Bhattacharya and Goldstein, Neuropharmacol. 9:109-118, 1970). We recently completed and reported a study of the effects of chronic nicotine administration on the electrical activity within and between brain structures of Sprague-Dawley rats which generally confirmed the earlier findings in rabbits (Nelsen, Pelley, and Goldstein, Res. Comm. Chem. Pathol. and Pharmacol. 5:694-704, 1973).

The proposed consequence of the electrophysiological changes was that behavior should become more specifically goal-oriented since, as Routtenberg suggested (Psychol. Rev. 75:51-80, 1968), the main functional significance of predominant limbic system mediation involves "incentive-oriented" arousal while that of the RF is "drive-oriented". Working under subsequent grants from The Council, we tested this proposal in rats rigorously trained so that they performed

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7.(b) at sustained, reproduceable levels on a difficult visual attention task. We found that chronic nicotine treatment did improve the efficiency of responses to goal-oriented stimuli above the control optimal levels without causing or being accompanied by a general, non-specific increase in behavioral activity (Nelsen and Goldstein, *Psychopharmacologia* 26:347-360, 1972). The results of these studies invited further research directed both towards a better understanding of the mechanisms and nature of arousal and of the motivations accounting for the widespread self-administration of nicotine by humans.

If as Routtemberg has suggested (and our results imply), there exists a balance between the limbic and RF influences on arousal, it would follow that modification of the relationships toward greater limbic system control (as in the "chronic nicotine state") should alter the sensitivity of the RF arousal pathway to manipulation. Our specific aims are to test this hypothesis directly using both electrophysiological and pharmacological tools. It is well demonstrated that electrical stimulation delivered to the RF produces cortical activation and that the current necessary to elicit the response varies depending on the state of the brain. We propose to characterize the changes in sensitivity of reticular-cortical relationships during the "chronic nicotine state" via studies of the threshold for and duration of cortical activation in chronically nicotine-treated and saline-treated rats. A number of stimulant and/or psycho-active drugs are known or

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7. (c) suspected to exert their CNS effects primarily by their actions at the level of the RF. Consequently, the alteration of cortical-limbic-reticular relationships resulting from chronic nicotine treatment should in turn modify the efficacy of these drugs. We have already conducted one study aimed at elucidating these effects by use of electroencephalographic measures.

(Please see the attached "Progress Report" for details.)

A further aim is to extend studies beyond the measurement of electroencephalographic effects at cortical and subcortical levels to include the detection of the behavioral or functional consequences of the electrophysiological and pharmacological manipulations. Because it has been demonstrated to be sensitive both to the level and qualitative nature of arousal, we propose to use the visual attention task described previously and reviewed in "item 9" to quantify the behavioral consequences of alterations in brain structure relationships.

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9. (a) The details of experimental design and procedures are essentially the same as those presented in our previous grant application (dated October 20, 1972) since that proposal was designed to include two years of research activity commencing January 1, 1973. The following reviews these experimental plans and includes the additions and changes we have incorporated. Two related lines of investigation are planned. The first concerns electroencephalographic measures of cortical activation induced by electrical stimulation via the mesencephalic reticular formation (RF) arousal pathway and by pharmacological agents. In previous applications for "Research Grants" from the Council (dated November 25, 1970 and October 25, 1971), we have reviewed the surgical and EEG measurement technics which we have developed for application to such studies. We propose to prepare with cortical and subcortical (RF) electrodes an experimental group of approximately 20 rats (Holtzman, Sprague-Dawley, male). These animals will undergo adaptation training in an EEG recording chamber. They will then be assigned in random fashion to two groups, one of which will receive chronic treatment with nicotine (100ug/kg, s.c., t.i.d.) and the other, with physiological saline.

A schedule for delivery of small doses of electrical stimulation to the RF will be carried out to determine the threshold for and the duration of cortical activation resulting from direct stimulation of the RF arousal pathway under conditions of chronic nicotine and chronic saline treatment. Arousal (activation) will be measured and quantified from the cortical EEG recordings. Further, comparisons between the

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9. (b) cortical effects of D-amphetamine, methyl phenidate, caffeine, physostigmine, pemoline, L.S.D., and tetrahydrocannabinol in animals treated chronically with either nicotine or saline will be made.

Electric current will be delivered by a pair of Grass stimulators which have been modified to obtain a reliable "constant current" output. Parameters of the current will be within a moderate range which has been shown to cause only reversible effects both on behavior and neural tissue (Kornetsky and Eliasson, 1969; Nelsen, 1970).

The second area of investigation involves behavioral measures of the effects of modifications in the proposed balance between limbic and RF control of arousal. Because in our hands it has proven to be impressively sensitive to levels of arousal, the same form of the behavioral task of Kornetsky and Elisson (1969) which we have described in previous grant applications to the Council (dated November 25, 1970 and October 25, 1971) will be used to assess the functional consequences of electrical and pharmacological manipulations of the RF arousal pathway.

Twelve rats will be prepared surgically with electrodes at the sensory-motor cortex and in the mesencephalic RF. Following recovery (about three weeks), these animals will be trained to perform on the visual attention task. The rats will be partially food deprived and maintained at approximately 85% of their normal, free-feeding body weights. They will be trained to press a lever for a food pellet reinforcement

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9. (c) following the presentation of a conditional stimulus (C.S.) which is the white cue light in a standard operant conditioning box. Training is carried out in a series of phases such that initially the task is quite elementary, i.e., continuous reinforcement or fixed ratio 1 in the presence of a constant C.S. During successive training sessions, an inter-trial interval (I.T.I.) is introduced and the duration of the C.S. is reduced in step-wise fashion while a punishment contingency for inappropriate responding is also added. In the task's final form, the duration of the cue light is only 0.2 sec, followed by an available response time of 5.0 sec during which only the first lever press is reinforced. Failure to press after a C.S. is scored as an omission error (o.e.) and has no consequence for the animal other than the loss of a reinforcement pellet. The I.T.I. is variable (so that the rat is not learning to time responses according to a fixed interval) with a mean of 10.0 sec. A lever press during the I.T.I. is scored as a commission error (c.e.) and is punished by the imposition of a 30.0 sec "time-out". Additional responses during the time-out reset the punishment clock to 30.0 sec and are also scored as c.e.'s. A session is terminated after 100 reinforcements have been delivered or after one hour has elapsed. The task is programmed via electro-mechanical modules.

This type of task in which the animal is asked not only to make appropriate responses but also to inhibit inappropriate responses is difficult for rats to learn and requires several months of training to achieve efficient performance which we

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8. (d) have defined as: 1) for o.e.'s, #o.e./# reinforcements times $100 \leq 30\%$, and 2) for c.e.'s, #c.e./# reinforcements times $100 \leq 30\%$. After the rats have reached criterion levels of performance, half the group will be subjected to chronic treatment with nicotine (100ug/kg, s.c., t.i.d.) and half to physiological saline. After approximately one week of regular injections, a schedule of low current level electrical stimulation to the RF will be introduced during the testing sessions. Such stimulation has been shown to disrupt performance in a similar task (Kornetsky and Eliasson, 1969) where electric foot-shock was the reinforcing agent. Since based on our previous work, differential baseline behavior is expected depending on whether rats are nicotine- or saline-treated, each group (in fact, each animal) will act as its own control. Our hypothesis is that because nicotine-treated rats are in a state of greater incentive-oriented arousal (hippocampal predominance in the control of cortical function), their performance will be affected less detrimentally by RF stimulation than the performance of saline-treated animals.

Further, behavioral studies are planned which will focus on the possible protective action of the chronic nicotine state on the disruptive effects of pharmacological agents known or suspected to act on the RF arousal pathway by causing a relative increase in general or drive-oriented arousal and hence, decrease in incentive-oriented arousal. These agents will include D-amphetamine, L.S.D., physostigmine, and tetrahydrocannabinol. They will be administered according to a randomized block

9. (e) design and the results will be tested by applying an analysis of variance.

The attached progress report includes the description of the study designed to test the electroencephalographic effects of acute stimulant or psycho-active drug "challenges" in rats treated chronically with either nicotine or saline. This study has been conducted as per the design presented and is presently under analysis.

Kornetsky, Conan and Eliasson, Mona: Reticular Stimulation and Chlorpromazine: An Animal Model for Schizophrenic Over-arousal. Science 165:1273-1274, 1969.

Nelsen, Judith M.: Single Dose Tolerance to Morphine Sulfate: Electroencephalographic Correlates in Central Motivational Systems. Unpublished Doctoral Dissertation. (Boston University) 1970.

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Curriculum VitaeLeonide Goldstein, D.Sc.

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Born:

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Marital Status:

REDACTED

- 1921-35 Student at the Conservatoire National de Musique, Paris
 1935 Graduated in violin, harmony and composition
 1935-36 Military duty, French Army
 1936-37 Technical Assistant Institut de Biologie, Paris
 1937-39 Research Assistant
 1939-40 Non-commissioned Officer, French Army
 1940 Undergraduate studies, University of Paris
 1941-42 Research Associate Laboratoire de Physiologie School of Medicine, University of Montpellier, France
 1942 Member of the Research Division of the Free French Forces
 1942-45 Special assistant to Dr. H.J. Muller, Amherst College, Amherst, Mass.
 1944 B.A. and M.A. Amherst College
 1945-47 Assistant Professor University of Paris (Physiology and Genetics)
 1947-53 Acting Director Laboratoire de Biometrie of the French National Research Council
 1951 Doctor of Sciences degree, University of Paris, Sorbonne
 1953-58 Assistant Professor, Neurophysiology, Ecole Pratique des Hautes Etudes, Sorbonne
 1958-61 Associate Professor, Pharmacology, Emory Univ., Atlanta, Ga.
 1961-64 Neuropharmacologist, Bureau of Research, Neuropharmacology Section, N.J. Neuropsychiatric Institute, Princeton, N.J.
 1964-72 Research Scientist Grade 1, Bureau of Research, Neuropharmacology Section, N.J. Neuropsychiatric Institute, Princeton, N.J.
 1969-73 Visiting Senior Fellow - Department of Biology - Princeton Univ.
 1972 Associate Professor of Psychiatry, Rutgers Medical School, Piscataway, N.J.
 1973 Member Graduate Faculty, Rutgers University, New Brunswick, N.J.
 1973 Member Psychobiology Area Graduate Program in Psychology, Rutgers University, New Brunswick, N.J.

Membership in Scientific Societies:**REDACTED****REDACTED****REDACTED**Honors:

Croix de Guerre (1939-40); Medal of the Free French Forces; Palmes Academiques (1950). Associate Editor "Research Communications in Chemical Pathology and Pharmacology."

Listings:

American Men at Science - Who is Who in the East.

Publications:

Author or co-author of 150 papers and abstracts.

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CURRICULUM VITAE

Judith M. Nelsen, Ph.D.

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BORN:

MARITAL STATUS:

REDACTED

- 1946-60 Primary and secondary studies, public schools of Town of Lake and City of Cudahy, Wisconsin
- 1960-63 Undergraduate studies, University of Wisconsin-Milwaukee (Letters and Science, Pharmacy)
- 1963 Laboratory assistantship in bacteriology (University of Wisconsin-Milwaukee)
- 1963-65 Undergraduate studies, University of Wisconsin- Madison (Pharmacy, Psychology)
- 1963-65 Research assistantship in physical chemistry (University of Wisconsin-Madison)
- 1964 Summer research assistantship in physical chemistry (from the U.S. Department of the Army at the University of Wisconsin-Madison)
- 1965 B.S. (HONORS) degree. University of Wisconsin. Madison, Wisconsin
- 1965-70 Graduate studies, Boston University School of Medicine, Division of Medical Sciences, Department of Pharmacology and Experimental Therapeutics (Major professor: Conan Kornetsky, Ph.D., Director, Laboratory of Behavioral Pharmacology)
- 1965-66 Graduate School Research Fellowship
- 1966-70 Public Health Service Research Fellowships. (N.I.M.H.)
- 1970 Doctor of Philosophy degree. Boston University. Boston, Mass.
- 1970-72 Post-doctoral appointment. N.J. Bureau of Research in Neurology and Psychiatry. Box 1000, Princeton, N.J.
- 1973 Senior Scientist. Rutgers Medical School. Department of Psychiatry, Piscataway, N.J.

HONORS: Sophomore Honors (U.W.); Senior Honors (U.W.); Sigma Epsilon Sigma (U.W.); Rho Chi (U.W.); Phi Kappa Phi (U.W.); Sigma Xi (B.U.)

PROFESSIONAL SOCIETY MEMBERSHIPS:

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PUBLICATIONS

Nelsen, Judith M. and Conan Kornetsky: Single Dose Tolerance to Morphine Sulfate: EEG Changes. *The Pharmacologist* 10: No. 2, 1968.

Weil, Andrew T., Norman E. Zinberg, and Judith M. Nelsen: Clinical and Psychological Effects of Marihuana in Man. *Science* 162: 1234-1242, 1968.

Nelsen, Judith M. and Leonide Goldstein: Improvement of Performance on an Attention Task with Chronic Nicotine Treatment in Rats. *The Pharmacologist* 13: No. 2, 1971.

Nelsen, Judith M. and Leonide Goldstein: Improvement of Performance on an Attention Task with Chronic Nicotine Treatment in Rats. *Psychopharmacologia* 26: 347-360, 1972.

Nelsen, Judith M. and Conan Kornetsky: Morphine-Induced EEG Changes in Central Motivational Systems: Evidence for Single-Dose Tolerance. Fifth International Congress on Pharmacology (Abstracts, p. 166, #993), 1972.

Goldstein, Leonide and Judith M. Nelsen: Some Views on the Neurophysiological and Neuropharmacological Mechanisms of Storage and Retrieval of Information. In: Memory and Transfer of Information (H.P. Zippel, ed.). Plenum Press, New York, 1973, pp. 155-191.

Nelsen, Judith M.: Neurophysiological and Behavioral Consequences of Chronic Nicotine Treatment. In: Drug Addiction, vol. III (J.M. Singh, L.H. Miller, H. Lal, eds.). Futura Publishing Co., Mount Kisco (N.Y.), 1973 (Chapter accepted for publication).

Nelsen, Judith M. and Leonide Goldstein: Chronic Nicotine Treatment in Rats: 1. Acquisition and Performance of an Attention Task. *Res. Comm. Chem. Pathol. and Pharmacol.* 5: 681-693, 1973.

Nelsen, Judith M., Kathleen Pelley, and Leonide Goldstein: Chronic Nicotine Treatment in Rats: 2. Electroencephalographic Amplitude and Variability Changes Occurring Within and Between Structures. *Res. Comm. Chem. Pathol. and Pharmacol.* 5: 694-704, 1973.

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